**PATENT CO-OPERATION TREATY,**

Australian Patent Office
PO Box 200, Woden Act 2606
Australia

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| Applicant | : | Biocon India Limited. |
| Application No. | : | PCT/IN2003/00383 |
| Int'l Filing Date | : | 5TH December 2003 |
| Title | : | Process for the purification of macrolides |
| Docket No. | : | 11352(PCT-59) |

**Amendment of description and claims under Article 34 (2) (b) PCT**

Australian Patent Office
PO Box 200, Woden Act 2606
Australia

Dear Sir,

Thank you very much for the written opinion dated 20th April 2004.

With regard to the Learned Examiner's observation that claim 1 is not clear due to the commencing word "novel", we have now deleted the said word.

We are enclosing herewith retyped pages in triplicate and request the Learned Examiner to issue a favourable International Preliminary Examination Report.

Yours faithfully,

Archana Shanker

Encl.: Retyped pages (in triplicate)

**(FACSIMILE & COURIER)**

**Our ref.: 11352(PCT-59)** **June 16, 2004**

Australian Patent Office
PO Box 200, Woden Act 2606
Australia
Fax # 02-6285-3929

**Re: Response to the Written Opinion**
**International Application No.: PCT/IN2003/00383**

Dear Sir,

Please find enclosed herewith our response to the Written Opinion dated 20th April 2004 in respect of the aforesaid international application.

Kindly take the said documents on record and acknowledge the receipt of the same.

With best regards,

Yours Sincerely,

Archana Shanker

Encl.: As Stated

We claim:

1. A process for the recovery of a macrolide in substantially pure form comprising:

a) treatment of an impure or crude macrolide with water immiscible solvent,

b) optional concentration of the mixture,

c) treatment with ammonia gas to phase out impurities,

d) separation of impurities,

e) optional concentration of the phase containing the macrolide,

f) loading on silica gel chromatography, optionally reversed phase or pretreated with silver, and elution of the macrolide,

g) affording the macrolide in substantially pure form,

h) optional repetition of step f and g to afford the macrolide in substantially pure form.

2. A process as in claim 1, wherein the macrolide is selected from tacrolimus, immunomycin or sirolimus.

3. A process as in claim 1, wherein the water immiscible solvent is selected from a group comprising hydrocarbons, heterocyclic compounds, ethers or esters.

4. A process as in claim 1, wherein the water immiscible solvents is selected from a group comprising benzene, toluene, hexane, ethyl acetate, isobutyl acetate or butyl acetate.

5. A process as in claim 1, wherein the macrolide compound is afforded by crystallization or precipitation.

TITLE OF THE INVENTION

# PROCESS FOR THE PURIFICATION OF MACROLIDES

FIELD OF THE INVENTION

This invention relates to a process for purification of macrolides.

BACKGROUND OF THE INVENTION

A compound, 15,19-Epoxy-3H-pyrido[2,1-c][1,4]oxaazacyclotricosine-1,7,20,21(4H,23H)-tetrone, 5,6,8,11,12,13,14,15,16,17,18,19,24,25, 26,26a-hexadecahydro-5,19-dihydroxy-3-[(1E)-2-[(1R,3R,4R)-4-hydroxy-3-methoxycyclohexyl]-1-methyl ethenyl]-14,16-dimethoxy-4,10,12,18-tetramethyl-8-(2-propenyl)-, (3S,4R,5S,8R,9E,12S,14S,15R,16S,18R,19R,26aS), also known as FK506 as well as tacrolimus disclosed by EP 184162 and US 4,894,366 is useful as an immunosuppressant. Another compound, 15,19-Epoxy-3H-pyrido[2,1-c][1,4]oxaazacyclotricosine-1,7,20,21 (4H,23H)-tetrone,8-ethyl-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26, 26a-hexadecahydro-5,19-dihydroxy-3-[(1E)-2-[(1R,3R,4R)-4-hydroxy-3-methoxycyclohexyl]-1-methylethenyl]-14,16-dimethoxy-4,10,12,18-tetramethyl-, (3S,4R,5S,8R,9E,12S,14S, 15R,16S,18R,19R,26aS)-, also known as immunomycin as well as FK 520, disclosed in EPO Publication No. 0184162 is also useful as an immunosuppressant. Many other derivatives of these compounds as well as structural analogues have immunosuppressant property.

**ABSTRACT**

The invention relates to a process for the recovery of a macrolide in substantial pure form comprising:

a) treatment of an impure or crude macrolide with water immiscible solvent,

b) optional concentration of the mixture,

c) treatment with ammonia gas to phase out impurities,

d) separation of impurities,

e) optional concentration of the phase containing the macrolide,

f) loading on silica gel chromatography, optionally reversed phase or pretreated with silver, and elution of the macrolide,

g) affording the macrolide in substantially pure form,

h) optional repetition of step f and g to afford the macrolide in substantially pure form.

The macrolide is preferably tacrolimus, immunomycin or sirolimus